Physiology & Behavior 94 (2008) 523–527

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Dissociation of the circadian rhythm of locomotor activity in a 22 h light–dark cycle impairs passive avoidance but not object recognition memory in rats

Sebastião Pacheco Duque Neto [a], Breno Tercio Santos Carneiro [a], Verónica Sandra Valentinuzzi [b], John Fontenele Araújo [a,*]

[a] Departamento de Fisiologia, Laboratório de Cronobiologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, CEP 59078-970, Natal-RN, Brazil
[b] CRILAR-CONICET, Entre Rios y Mendoza s/n, 5301, Anillaco, La Rioja, Argentina

ARTICLE INFO

Article history:
Received 22 November 2007
Received in revised form 24 March 2008
Accepted 25 March 2008

Keywords:
Circadian rhythm
Dissociation
Learning and memory
Rat

ABSTRACT

We analyzed the effect of dissociation of the circadian rhythm of locomotor activity on the performance in two memory tasks in rats. One group of animals was maintained in a normal 24 h light–dark cycle of 12:12 (T24 group, control). A second group was housed in a 22 h cycle of 11:11 (T22 group, experimental), a condition which is known to produce dissociation of the circadian rhythm of locomotor activity in two components. Both groups were tested on two memory tasks: passive avoidance and object recognition. An additional control group, kept under constant darkness (DD group), was used for a passive avoidance task. Testing occurred 30 min (short-term memory — STM) and 24 h (T24 and DD group) or 22 h (T22 group) (long-term memory — LTM) after training. The T22 group showed impairment on the passive avoidance task (STM and LTM) compared with the T24 and DD groups. On the object recognition task, the T22 and T24 groups performed similarly in all the sessions. In conclusion, circadian rhythm dissociation induced a performance deficit in the passive avoidance task but had no effect on the object recognition task. We suggest that dissociation of the circadian rhythm of locomotor activity may selectively affect some emotional component related to fear and risk evaluation.

1. Introduction

The suprachiasmatic nuclei of the hypothalamus, the master structure of the mammalian circadian timing system, are thought to be a population of coupled circadian oscillators [1,2]. Knowledge of the coupling mechanism is essential to understand the expression of the circadian output and to develop new treatments for circadian rhythm sleep disorders (CRSDs; e.g., jet lag, shift work sleep disorder, delayed sleep phase syndrome, advanced sleep phase syndrome, non-24 h sleep–wake syndrome) [3].

Disruptions of circadian rhythms are known to affect performance negatively. For example, cognitive deficits have been observed in subjects submitted to jet lag [4,5], to shift work schedules [6–8], with CRSDs [3], with chronic sleep pattern disorganization [9] as well as age-related circadian disruption [10]. This has been further confirmed in laboratory studies where significant performance deficits are observed in subjects submitted to frequent delays and advances of the LD cycle [11,12]. Additionally, when rhythmicity is normal, performance in diverse learning tasks has been shown to vary according to the time of training and testing [13–17] suggesting a relationship between the circadian system and structures involved in cognitive processes.

It has been proposed that coupling within the SCN may be due to gap junctions and/or to a GABA-dependent mechanism [18–22]. Whatever the case, the degree of coupling seems to be a key factor in determining the overt rhythm pattern. If coupling is low, then the oscillators may easily be dissociated and ultradian rhythms or arrhythmic behavior may be generated, as occurs in developing animals or from the effect of constant light [23,24]. If coupling is strong, then a stable circadian rhythm is manifested. In between, some abnormal patterns in rhythm behavior may appear under certain experimental conditions, such as splitting, in which two circadian rhythms may appear simultaneously [25,26], or under 4 h light–dark (LD) cycle, when six circadian components manifest [27]. These patterns may be due to partial coupling of the circadian system, when there are at least two functional groups of oscillators, each with a different period. Moreover, rats maintained in a symmetric 22 h LD cycle express two simultaneous components of locomotor activity rhythm, with different periods [28]. One component is entrained to the external cycle (Light Dependent Component — LDC), whereas the

Abbreviations: LD, light–dark; DD, constant darkness; LDC, light dependent component; NLDC, non-light dependent component; SCN, suprachiasmatic nucleus; STM, short-term memory; LTM, long-term memory; GABA, γ-aminobutyric acid.

* Corresponding author. Universidade Federal do Rio Grande do Norte (UFRN), Departamento de Fisiologia, Centro de Biociências, Campus Universitário, Lagoa Nova, Caixa Postal 1506, CEP: 59078-970, Natal-RN, Brazil. Tel.: +55 84 3215 3409x218; fax: +55 84 3211 9206.
E-mail address: araujo@cb.ufrn.br (J.F. Araújo).

0031-9384/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2008.03.013

other free runs with a period greater than 24 h (Non-Light Dependent Component — NLDC). Since the circadian clock in the suprachiasmatic nucleus of the hypothalamus (SCN) contains multiple autonomous single-cell circadian oscillators, we tend to interpret the expression of two circadian components in T22 LD, as being caused by the reduced degree of coupling between the oscillators that drive the circadian system [29]. Studying *Per1* and *BMAL1* expression, de la Iglesia et al. [29] showed that in rats under T22, the ventrolateral and dorsomedial portions of SCN become desynchronized with each other. The activity of the ventrolateral portion is associated with LDC, while the NLDC is related to the dorsomedial activity portion. It has been proposed that the expression of these two components is dependent on light intensity and physical exercise [27,30]. Recently, Cambras et al. [31] have reported that in desynchronized rats, the body temperature and paradoxical sleep rhythms could be dissociated from those of rest–activity, sleep–wake, and slow-wave sleep.

A partial rather than whole-structure entrainment of the multi-oscillatory system may explain this gradual appearance of two components, such that some oscillators may entrain while others may free-run [32]. However, we cannot totally rule out that internal desynchronization, both in this model and in other models, could involve dissociation between SCN and non-SCN oscillators, instead of or in addition to intra-SCN dissociations.

It is not known whether the spontaneous and forced internal desynchronization of physiological and behavioral rhythms observed in humans represents the activity of two independent oscillators and, if it does, whether these oscillators are anatomically identifiable [31]. In addition, the lack of animal models has slowed progress toward understanding the pathophysiology associated with circadian desynchronization.

We suggest that the dissociation of circadian activity rhythm under T22 could be a good animal model to investigate the pathophysiology, and thus determine possible approaches for managing circadian rhythm sleep disorders. Here, we present results of learning performance of rats with dissociation of the circadian rhythm of locomotor activity using two different learning tasks, one which is considered to have a strong emotional component (passive avoidance) and another considered to be less emotional (object recognition).

2. Materials and methods

2.1. Animals and housing

Twenty eight male Wistar rats from our own breed, 4–7 months old, weighing 312–450 g were used. Animals were coupled-housed in standard polypropylene cages (32×17×40 cm) with food (Purina®) and water *ad libitum*. Cages were placed in light-tight, ventilated wooden cabinets (180×55×50 cm) with timer control for lighting conditions and constant temperature (24±2 °C). The LD cycle varied according to the experimental groups as explained below; light provided by two 40-W fluorescent bulbs, 200 lx at the cage lid level. Eight animals were submitted to a 24 h (12 h:12 h) light–dark cycle (T24 group), twelve to a 22 h (11 h:11 h) light–dark cycle (T22 group) and an additional control group (n=8), maintained under constant darkness (DD group). General locomotor activity was constantly measured with a homemade computer program using infrared motion sensors (Focus 2000, *Aspex* ®) localized 15 cm above the cage lids. Experiments were in compliance with the institutional guidelines of the Universidade Federal do Rio Grande do Norte and Sociedade Brasileira de Neurociência e Comportamento.

2.2. Experimental procedures

Animals from all the groups were submitted to the memory tasks in the middle of their active phases. For this, the day before behavioral testing, individual actograms were analyzed and time for testing was determined. In the case of the T22 group, the tasks were performed only when the active phase of the NLDC component overlapped with the same phase of the LDC component. With this protocol we assured that the three groups were trained and tested at the same circadian phase. All learning sessions lasted 5 min.

Memory was evaluated using two tasks: step down passive avoidance and object recognition. The passive avoidance chamber (20 cm high, 25 cm wide and 21 cm deep) consisted of two lateral aluminum walls, a back wall, a transparent acrylic roof and a black acrylic front door. A grid floor made of stainless steel rods (0.5 cm diameter) 1 cm apart was connected to a shock generator (Ampere Ltda, Brazil). A wooden escape platform (20×5×2 cm) was positioned at the bottom of the chamber. During training, the animals received a 2 s, 0.5 mA scrambled foot shock each time they descended from the platform. Memory retention was evaluated 30 min (short-term memory — STM) and 24 h (T24 and DD group) or 22 h (T22 group) after training (long-term memory — LTM). This 22 h interval for the T22 group was used so that testing would occur exactly one cycle after training and consequently coincide with the middle of the dark phase. Test sessions consisted of measuring the latency it took each animal to step down from the platform and place their four paws on the grids. Approximately one week later the same animals were submitted to the object recognition task. During training, the subject was placed in a wooden open field (50×50×50 cm) in the presence of two identical side-by-side objects (two white tarnished porcelain inverted bowls) designated A1 and A2. Test 1, in which a novel object (B; white plastic rectangle with irregular surface) replaced one of the identical objects, occurred 90 min later (STM). In test 2, 22 or 24 h after training (LTM), a new object (C; white smooth shiny porcelain inverted cup) replaced B. Contact frequency and exploration time of each object were registered. A recognition index was calculated for both these variables as the rate between frequency or time exploring one object and the sum of both objects. That is, time A1/(time A1+time A2) for training, time B/(time B+time A) for STM and time C/(time C+time A) for LTM. The same calculation was made for frequency. Values near 1 indicate that the animal explored the novel object more. Performance was considered good when exploration of the novel objects (B or C) was significantly higher than that of the known object (A).

All trials were videotaped and the behaviors were subsequently scored by an experimenter unaware of the treatments. Light intensity during sessions was approximately 30 lx at the floor level of each apparatus. After each session the apparatus was cleaned with alcohol 46%.

2.3. Data analysis

Output from the sensors was integrated with IBM-compatible homemade data acquisition software. Graphical output (actograms) and rhythm analysis were carried out using *El Temps* software (A. Díez-Noguera, Universitat de Barcelona, 1999). Periodicity of the activity rhythm was estimated using the original method described by Sokolove and Bushell [33], with a global risk level (α) of $P<0.05$.

T22, T24 and DD groups were compared statistically using an unequal variance t-test for escape latency in passive avoidance. For the recognition index, a paired sample statistic t-test was used for comparisons within the groups and an unequal variance t-test between the groups. Data are expressed as means±standard error mean (S.E.M).

3. Results

Fig. 1 shows the actograms and periodograms of representative animals of each of the three groups. As expected, under a T24 cycle, animals where normally entrained showing a 24 period while rats in DD exhibited a free-running rhythm of 1453.5±2.1 min. Finally, animals under the atypical T22 cycle presented dissociation of the locomotor activity rhythm in two components, one with a period of 1320 min and another of 1480 to 1505 min. Note that the amplitude of

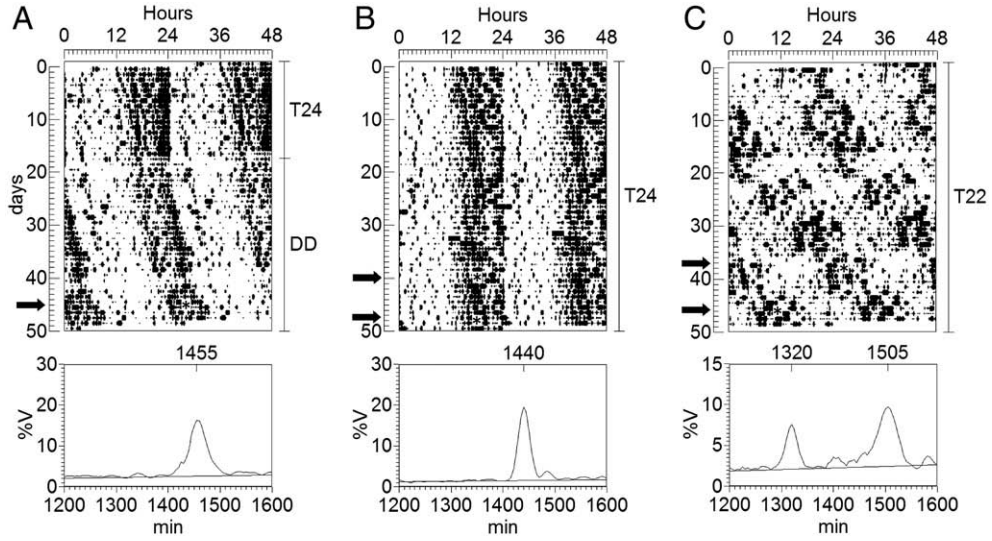

Fig. 1. Actograms (top) and periodograms (bottom) of representative animals from the DD (A), T24 (B) and T22 (C) groups. As indicated by black horizontal arrows and asterisks, testing occurred on days 45 and 46 for the DD group; passive avoidance task was performed on days 38–39 for the T22 group and 40–41 for the T24 group; object recognition task was performed five days later, on days 46–47, for the T22 group and 9 days later, on days 48–49, for the T24 group.

the LDC components is reduced, suggesting weak entrainment, nevertheless the periodogram clearly detected two rhythmic components.

Latency to step down in the passive avoidance short-term and long-term memory test is shown in Fig. 2. Training sessions are not showed because latency to step down in this session was basically inexistent. In other words, the drive to explore of both groups was similar. Clearly, animals under T22 had worse performance in both test sessions of passive avoidance when compared to the T24 and DD groups. T22 animals took 54.8±33.1 (STM) and 57.6±30.0 (LTM) seconds to step down while T24 took 262.8±37.1 (STM) and 204.9±48.6 (LTM) and DD animals 225.3±48.8 (STM) and 188.1±54.5 (LTM) seconds. That is, animals under T22 show a performance deficit in this aversive task suggesting some type of deficit in memory acquisition, consolidation or retrieval or in some other related process.

Fig. 3 shows performance in the object recognition task during training and both testing sessions of T24 and T22 groups. Even though both groups showed good short-term memory performance expressed by significantly higher recognition indexes relative to the training sessions, no significant differences were observed between groups. The similar performance of T22 and T24 animals in this task could be considered as a strong indication that dissociation did not affect sensory-motor functions such as locomotion or other variables such as motivation to explore. In all sessions (training, STM and LTM), the groups showed similar recognition indexes for frequency and exploration time. In other words, dissociation did not appear to affect any parameter of the object recognition task. The fact that during training, frequency of contacts with both objects (A1 and A2) was similar in both groups suggests a similar drive for exploration. In other words, as in the case of similar latency level during training of passive avoidance, this can be considered an indicative that the drive for exploration was not altered in the T22 group.

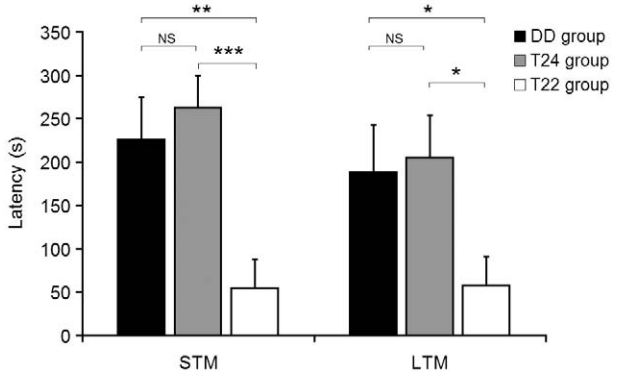

Fig. 2. Latency to step down from the platform during short- and long-term memory tests for each of the three groups (DD, T24 and T22). Data are expressed as means±S.E.M. *P<0.05, **P<0.01, ***P<0.001. NS: non-significant.

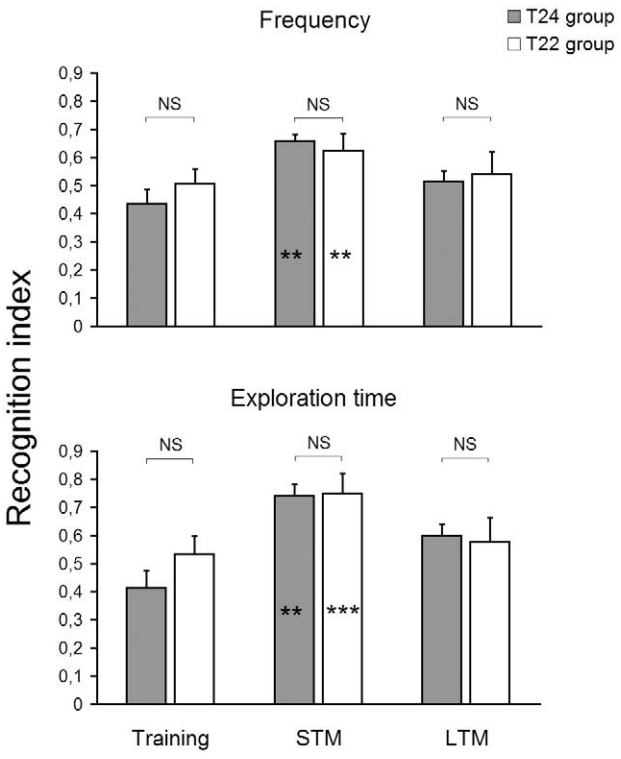

Fig. 3. Recognition index for frequency (top) and exploration time (bottom) during short-term and long-term memory testing for groups T24 and T22. Asterisks indicate comparisons within the groups between STM testing and training. **P<0.01, ***P<0.001. NS, non-significant. Recognition indexes of LTM were not statistically different from training.

Other than the differential effect in the step down latencies during both passive avoidance tests (STM and LTM), we did not detected any other change in the general behavior of the three groups, neither during the learning sessions nor in their home cages nor during handling.

4. Discussion

Forced desynchronized animals expressed impairment in their performance in a learning task when compared to animals synchronized to a normal LD cycle as well as animals free-running in DD. Latency to step down during both passive avoidance tests for short-term and long-term memory where significantly smaller compared to both control groups. On the other hand, performance in the object recognition task was apparently not affected by the circadian disorganization induced by the T22 cycle.

Many mechanisms that may explain our data can be discussed. First of all, the decreased passive avoidance performance in animals under T22 could be due to an increase in the motor activity level of these animals that could lead to lower step down latencies. However, some evidence suggests that this was not the case since, 1) during the object recognition test, T22 and T24 animals did not show differences in exploratory activity, 2) a correlation analysis between motor activity and latency values in the passive avoidance task was not significant (data not shown) and, 3) the literature shows that activity levels are not altered in forced desynchronized animals [27]. It could also be argued that animals with dissociation of the circadian rhythm have decreased sensibility and consequently a lower response to shock. We believe this is not the case since we noticed no difference in the animals' response level to the shock or any other behavioral change. Moreover, the normal exploration level and general performance in the object recognition task suggests that the sensorial system of these animals was working normally.

Sleep deprivation could be causing the aversive memory deficit in animals under T22. However, it is well documented that sleep deprivation negatively affects object recognition memory [34], which was not the case in our animals. Furthermore, recent data [31] suggest that these animals are not sleep-deprived, despite slow-wave sleep exhibiting two occurrence components (one entrained to the LD cycle and the other in free-running), whereas paradoxical sleep shows only a free-running component. In other words, sleep deprivation seems not to be the cause of worse performance on the passive avoidance task in animals under T22. Another issue that could explain these results is the increased stress level of animals under T22 with elevated corticosterone levels that could lead to memory impairment. Again, we do not believe this is the case because previous studies have shown that increased corticosterone levels impair object recognition memory [35] which, again, was not the case here. We can also argue that the animals were in T22 for a long period before the tests (almost 40 days), probably allowing an adaptation to these conditions. This is further supported by the fact that we did not detect any evidence of stress or discomfort in the animals. Another possibility is that the memory deficit related to fear and risk evaluation is simply due to the non-entrainment to a normal cycle of 24 h or a reduction in the amplitude of the rhythm. However, the control animals in free-running conditions, where the amplitude is reduced, performed very similarly to animals under T24, although a stable rhythm was expressed and coupling was strong.

Another issue to consider is that even though the 22-hour LD model has been evaluated in a series of articles as an animal model of internal desynchronization among distinct circadian oscillators, the relative contributions of entrainment and masking effects to the 22-hour component in this model are not well understood [36]. In this sense, we should keep in mind that, if the LDC component was in fact the result of pure masking, meaning that the endogenous rhythms is free-running, it could be argued that our training and testing sessions occurred in random circadian phases. Since performance in many tasks is known to change from one phase to another, results from T22 could not be compared with those of T24. However, we can discard this problem due to the fact that training and testing in the T22 animals only occurred in the middle of the active phase when the active phase of both components overlapped. In this sense, we can be sure that animals of all three groups where tested in the same circadian phase.

Additionally, since both learning tasks were performed in the same animals, it could be argued that the sequence of the tasks could interfere with the results. We cannot rule out the possibility that the passive avoidance task affected in one way or another performance in the object recognition task which occurred afterwards. Considering that the first task involves an electrical shock, that is a stressful situation, we would expect that if there should be an effect on the second task it would be towards a decreased performance, low exploration due to the memory of the shock in the first task as well as other stress symptoms. Since performance in the recognition task was normal, and exploration as well as activity level was good, and, furthermore, no difference was observed between groups, we believe that the sequence used in the present experiment did not affect performance. Also, probably one week between tasks was enough time to recover from any negative effect of the first task.

Under T22, the amplitude of two rhythmic components was reduced, meaning that the LDC is a weak entrainment. Even though we cannot conclude that these two components are explained only by rhythms generated in two distinct ensemble oscillators in the SCN, we can conclude that under T22 there is a break in the circadian system, and that these two behavioral components represent internal desynchronization, at least in the behavior domain. We are supposing that the memory deficit observed in T22 animals is best explained by the fact that the animals suffered from internal desynchronization of the circadian system. The role of the circadian system in memory consolidation and performance is not well understood however, clearly, a strong relationship exists between performance in learning tasks and the circadian system. Some data indicate that acquisition, recall, and extinction of associative learning can be modulated by circadian processes [13,15]. Furthermore, subjects submitted to circadian disorganization of diverse causes (jet lag, shift work, experimental phase shifts, aging, and altered sleep patterns) always express a decrease performance in diverse learning tasks [4,5,7,10–12].

In our animal model, passive avoidance but not object recognition memory was impaired. That is, we are suggesting the participation of the circadian system in some emotional component of the task that could be the acquisition and/or consolidation of memories related to fear and risk evaluation or alternatively simply a change in the emotionality of the forced desynchronized animals which in turn affects performance. Maybe animals under T22 are less emotional and consequently a painful shock is not enough to overcome the natural drive to explore. In other words, the animal may perfectly well remember that if it steps down it will receive a shock but simply does “not care” about it.

In this sense, the neural substrate that has been related to performance in aversive conditioning tasks is the amygdaloid complex [37–41]. It is interesting to mention that there is an indirect anatomic pathway between the SCN and the amygdala [42]. The central and basolateral nucleus of the amygdala exhibit opposite diurnal rhythms of clock Period2 protein expression, with such expressions becoming arrhythmic when the SCN is lesioned [43]. We should also keep in mind that emotionality has been shown before to be affected by circadian rhythm disruption; for example, negative effects on mood are common in humans [44].

In current society, many individuals have abnormal sleep–wake schedules and consequent internal desynchronization because of their occupations (e.g., shift-workers, transmeridian travelers). This rhythmic alteration occurs because most workers do not adapt to working at night and sleeping during the day. Recently, Wright et al. [45]

reported learning impairment in humans under internal desynchronization conditions, that is, a sleep–wake cycle out of phase with the internal biological time. As a consequence of internal desynchronization, some economic costs may arise as a result of work accidents or reduced productivity [46]. Therefore, understanding the mechanisms underlying internal desynchronization and its consequences is essential for human health and society. The animal model of forced desynchronization under T22 can help in this regard.

Acknowledgements

This research was supported by CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior*), CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*), FINEP (Financiadora de estudos e Projetos), MCT (Ministério da Ciência e tecnologia) and FAPERN (*Fundação de Amparo à Pesquisa do Estado de Rio Grande do Norte*).

We are grateful to José Demetrio Paliza for his constant and unselfish technical support.

References

[1] Welsh DK, Logothetis DE, Meister M, Reppert SM. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. Neuron 1995;14:697–706.
[2] Honma S, Shirakawa T, Katsuno Y, Namihira M, Honma K. Circadian periods of single suprachiasmatic neurons in rats. Neurosci Lett 1998;250:157–60.
[3] Scheer FA, Czeisler CA. Melatonin, sleep, and circadian rhythms. Sleep Med Rev 2005;9:5–9.
[4] Cho K, Ennaceur A, Cole JC, Suh CK. Chronic jet lag produces cognitive deficits. J Neurosci 2000;20(RC66):1–5.
[5] Cho K. Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. Nat Neurosci 2001;4:567–8.
[6] Rajaratnam SMW, Arendt J. Health in a 24-h society. Lancet 2001;358:999–1005.
[7] Rouch I, Wild P, Ansiau D, Marquié JC. Shiftwork experience, age and cognitive performance. Ergonomics 2005;48:1282–93.
[8] Rosekind MR. Underestimating the social costs of impaired alertness: safety, health and productivity risks. Sleep Med 2005;6:S21–5.
[9] Medeiros ALD, Mendes DBF, Lima PF, Araújo JF. The relationships between sleep–wake cycle and academic performance in medical students. Biol Rhythm Res 2001;32:263–70.
[10] Antoniadis EA, Ko CH, Ralph MR, McDonald RJ. Circadian rhythms, aging and memory. Behav Brain Res 2000;114:221–33.
[11] Tapp WN, Holloway FA. Phase shifting circadian rhythms produces retrograde amnesia. Science 1981;211:1056–8.
[12] Fekete M, van Ree JM, Niesink RJ, de Wied D. Disrupting circadian rhythms in rats induces retrograde amnesia. Physiol Behav 1985;34:883–7.
[13] Valentinuzzi VS, Kolker DE, Vitaterna MH, Ferrari EAM, Takahashi JS, Turek FW. Effect of circadian phase on context and cued fear conditioning in C57BL/6j mice. Anim Learn Behav 2001;29:133–42.
[14] Ralph MR, Ko CH, Antoniadis EA, Seco P, Irani F, Presta C, McDonald RJ. The significance of circadian phase for performance on a reward-based learning task in hamsters. Behav Brain Res 2002;136:179–84.
[15] Chaudhury D, Colwell CS. Circadian modulation of learning and memory in fear-conditioned mice. Behav Brain Res 2002;133:95–108.
[16] Valentinuzzi VS, Menna-Barreto L, Xavier GF. Effect of circadian phase on performance of rats in the Morris water maze task. J Biol Rhythms 2004;19:312–24.
[17] Valentinuzzi VS, Neto SPD, Carneiro BTS, Santana KS, Araújo JF, Ralph MR. Memory for time of training modulates performance on a place conditioning task in marmosets. Neurobiol Learn Mem 2008;89:604–7, doi:10.1016/j.nlm.2007.08.002.
[18] Colwell CS. Rhythmic coupling among cells in the suprachiasmatic nucleus. J Neurobiol 2000;43:379–88.
[19] Long MA, Jutras MJ, Connors BW, Burwell RD. Electrical synapses coordinate activity in the suprachiasmatic nucleus. Nat Neurosci 2005;8:61–6.
[20] Liu C, Reppert SM. GABA synchronizes clock cells within the suprachiasmatic circadian clock. Neuron 2000;25:123–8.
[21] Albus H, Vansteensel MJ, Michel S, Block GD, Meijer JH. A GABAergic mechanism is necessary for coupling dissociable ventral and dorsal regional oscillators within the circadian clock. Curr Biol 2005;15:886–93.
[22] Carpentieri AR, Pujoràs MA, Chiesa JJ, Noguera AD, Cambras T. Effect of melatonin and diazepam on the dissociated circadian rhythm in rats. J Pineal Res 2006;40:318–25.
[23] Canal-Corretger MM, Cambras T, Vilaplana J, Díez-Noguera A. Bright light during lactation alters the functioning of the circadian system of adult rats. Am J Physiol Regul Integr Comp Physiol 2000;278:R201–8.
[24] Canal-Corretger MM, Vilaplana J, Cambras T, Díez-Noguera A. Effect of light on the development of the circadian rhythm of motor activity in the mouse. Chronobiol Int 2001;18:683–96.
[25] Boulos Z, Terman M. Splitting of the circadian rhythm in the rat. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 1979;134:75–83.
[26] Earnest DJ, Turek FW. Splitting of the circadian rhythm of activity in hamsters: effects of exposure to constant darkness and subsequent re-exposure to constant light. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 1982;145:405–11.
[27] Vilaplana J, Cambras T, Díez-Noguera A. Dissociation of motor activity circadian rhythm in rats after exposure to LD cycles of 4-h period. Am J Physiol Regul Integr Comp Physiol 1997;272:R95–R102.
[28] Campuzano A, Vilaplana J, Cambras T, Díez-Noguera A. Dissociation of the rat motor activity rhythm under *T* cycles shorter than 24 hours. Physiol Behav 1998;63:171–6.
[29] de la Iglesia HO, Cambras T, Schwartz WJ, Díez-Noguera A. Forced desynchronization of dual circadian oscillators within the rat suprachiasmatic nucleus. Curr Biol 2004;14:796–800.
[30] Cambras T, Vilaplana J, Campuzano A, Canal-Corretger MM, Carulla M, Díez-Noguera A. Entrainment of the rat motor activity rhythm: effects of the light–dark cycle and physical exercise. Physiol Behav 2000;70:227–32.
[31] Cambras T, Weller JR, Anglès-Pujoràs M, Lee ML, Christopher A, Díez-Noguera A, Krueger JM, de la Iglesia HO. Circadian desynchronization of core body temperature and sleep stages in the rat. Proc Natl Acad Sci U S A 2007;104:7634–9.
[32] Vilaplana J, Cambras T, Campuzano A, Díez-Noguera A. Simultaneous manifestation of free-running and entrained rhythms in the rat motor activity explained by a multioscillatory system. Chronobiol Int 1997;14:9–18.
[33] Sokolove PG, Bushell WN. The chi square periodogram: its utility for analysis of circadian rhythms. J Theor Biol 1978;72:131–60.
[34] Palchykova S, Winsky-Sommerer R, Meerlo P, Durr R, Tobler I. Sleep deprivation impairs object recognition in mice. Neurobiol Learn Mem 2006;85:263–71.
[35] Okuda S, Roozendaal B, McGaugh JL. Glucocorticoid effects on object recognition memory require training-associated emotional arousal. Proc Natl Acad Sci USA 2004;101:853–8.
[36] Cambras T, Chiesa J, Araújo J, Díez-Noguera A. Effects of photoperiod on rat motor activity rhythm at the lower limit of entrainment. J Biol Rhythms 2004;19:216–25.
[37] Vazdarjanova A, McGaugh JL. Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. J Neuroci 1999;19:6615–22.
[38] Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 2000;406:722–6.
[39] Barros DM, Ramirez MR, Izquierdo I. Modulation of working, short- and long-term memory by nicotinic receptors in the basolateral amygdala in rats. Neurobiol Learn Mem 2005;83:113–8.
[40] Herdade KC, Strauss CV, Zangrossi Júnior H, Vianna MB. Effects of medial amygdala inactivation on panic-related behavior. Behav Brain Res 2006;172:316–23.
[41] Canal CE, Chang Q, Gold PE. Amnesia produced by altered release of neurotransmitters after intraamygdala injections of a protein synthesis inhibitor. Proc Natl Acad Sci USA 2007;104:12500–5.
[42] Peng ZC, Bentivoglio M. The thalamic paraventricular nucleus relays information from the suprachiasmatic nucleus to the amygdala: a combined anterograde and retrograde tracing study in the rat at the light and electron microscopic levels. J Neurocytol 2004;33:101–16.
[43] Lamont EW, Robinson B, Stewart J, Amir S. The central and basolateral nuclei of the amygdala exhibit opposite diurnal rhythms of expression of the clock protein Period2. Proc Natl Acad Sci USA 2005;102:4180–4.
[44] Parkes RP. Age, smoking, and negative affectivity as predictors of sleep patterns among shiftworkers in two environments. J Occup Health Psychol 2002;7:156–73.
[45] Wright KP, Hull JT, Hughes RJ, Ronda JM, Czeisler CA. Sleep and wakefulness out of phase with internal biological time impairs learning in humans. J Cogn Neurosci 2006;18:508–21.
[46] Akerstedt T. Shift work and disturbed sleep/wakefulness. Sleep Med Rev 1998;2:117–28.